**T355 Effect of sample processing on in situ organic matter degradability of distillers grains.** M. L. Drewery*[1], J. E. Sawyer[1], N. M. Kenney[1], W. E. Pinchak[2], and T. A. Wickersham[1], *[1]Texas A&M University, College Station, [2]Texas AgriLife Research, Vernon.*

Determination of ruminal OM degradability is important when evaluating feedstuffs. Precise quantification of nutrient availability is important when formulating rations. Wet distillers grains (DG) have a high moisture content that challenges conventional sample processing methods. Our objective was to quantify the effect of sample processing on measures of rate and extent of OM degradation of wet DG samples. Three ruminally cannulated steers were given ad libitum access to a ration (15% CP) containing 38.5% corn, 28% hay, and 28% dried DG. Samples of DG from each plant were divided and a portion was frozen at −20°C while the remainder was dried at 55°C in a forced-air oven for 96 h. Dried samples were ground to pass a 2-mm screen. Five g of each sample was placed in Dacron bags, pre-incubated in tepid water, placed in a weighted mesh polyester bag, and incubated in the rumen for 4, 6, 12, 24, 48 and 72 h. Samples were rinsed in cold water and dried at 60°C. Organic matter was measured and fractionated into A, B, and C pools. Degradation rate of the B fraction was calculated as the slope of the natural log of N remaining against time. Rate of passage was set at 3%/h. The A fraction was larger ($P < 0.01$) for frozen than dried samples, 43.5 and 33.1%, respectively. In contrast, the B fraction was less ($P < 0.01$) for frozen (42.9%) than dried samples (52.6%). The C fraction was not significantly different ($P = 0.16$) between frozen (13.6%) and dried (14.4%). Similarly, the difference in the degradability of the B fraction was not significant ($P = 0.71$) for frozen than dried samples 5.38 and 5.57%/h, respectively. However, estimated degradability was observed to be greater ($P < 0.01$) for frozen than dry samples 69.2 and 65.4%, accordingly. Our observations suggest sample processing affects the fractionation of OM from DG, but not estimates of OM degradability.

**Key words:** distillers grains, degradability, organic matter

**T356 Effect of tannins on in vitro ruminal degradability of purple prairie clover (*Petalostemon purpureum*) harvested at the two growth stages.** L. Jin*[1,2], Z. Xu[1], A. D. Iwaasa[3], Y. G. Zhang[2], M. P. Schellenberg[3], T. A. McAllister[1], and Y. Wang[1], *[1]Agriculture and Agri-Food Canada, Lethbridge Research Centre, Lethbridge, AB, Canada, [2]Department of Animal Science, Northeast Agricultural University, China, [3]SPARC-AAFC, Swift Current, SK, Canada.*

An in vitro study was conducted to assess the effects of maturity and tannins on the ruminal degradability of purple prairie clover (PPC; *Petalostemon purpureum*).Whole PPC plants were harvested from 3 pastures at the vegetative (VEG) and full-flowering/early seeding (FL) stages. Ground whole plant samples were placed into ANKOM F57 filter bags that were then incubated with a mixture of ruminal fluid and buffer in glass digestion jars in 3 DAISYII fermentors. Ruminal fluid was collected from steers fed forage (barley silage/grain/alfalfa hay) diet. $^{15}N$ labeled ammonium sulfate was included in the inoculum to assess microbial protein synthesis and feed colonization. Filter bags that contained the samples collected from the same pasture at the 2 growth stages were incubated in a same unit. Half (2) of the jars in each unit were supplemented with polyethylene glycol (PEG), yielding a 2 × 2 arrangement in each unit. Two bags were retrieved from each jar at 0, 1, 2, 4, 8, 12, 24, 48 and 72 h of incubation. All bags withdrawn were washed under running tap water until the water was clear, dried, and analyzed for DM, N and $^{15}N$. Plants harvested at the VEG stage had higher ($P < 0.001$) true dry matter degradability (TDMD), total N degradability (TND) and potential degradable fraction (b) of DM and N than that harvested at FL stage. The rapidly degradable fraction (a) of DM was higher ($P < 0.001$) in VEG than in FL, whereas the same fraction of N was higher ($P < 0.01$) in FL than in VEG. Inclusion of PEG increased ($P < 0.01$) the rate at which b is degraded (c) for DM only, but no growth stage by PEG interaction was found for DM degradation parameters. Inclusion of PEG increased ($P < 0.01$) TND and the potentially degradable fraction of N of PPC harvested at the FL, but not at the VEG stage. Overall, the results indicated that PPC harvested at the VEG stage is more degradable in the rumen than that harvested at the FL stage, but it seemed that the high tannins concentrations had only inferiorly detrimental impact on PPC degradability.

**Key words:** purple prairie clover, tannins, ruminal degradability

**T357 Effect of exogenous fibrolytic enzymes on dry matter in situ digestibility of two *Brachiaria* grasses.** J. H. Avellaneda-Cevallos[1,2], O. D. Montañez-Valdez*[3], D. Romero-Garaicoa[1], R. Luna-Murillo[1], J. Bravo-Loor[1], and M. Peña-Galeas[1], *[1]Unidad de Investigacóin Científica y Tecnológica. Facultad de Ciencias Pecuarias. Universidad Técnica Estatal de Quevedo, Quevedo, Ecuador, [2]Jefatura de Investigacóin. Carrera de Pecuaria.Escuela Superior Politécnica Agropecuaria de Manabí Manuel Félix López. Campus Politécnico, Sitio El Limón, Calceta, Manabí, Ecuador, [3]Centro Universitario del Sur de la Universidad de Guadalajara, Ciudad Guzmán, Jalisco, México.*

The effect of an exogenous fibrolytic enzymatic compound on ruminal pH and in situ digestibility of 2 *Brachiaria* hays, *B. mulato* and *B. decumbens*, cut at 28 and 56 d was evaluated. Four ruminally cannulated steers (400 ± 15 kg body weight) were randomly assigned to a replicated 4 × 4 Latin square with 2 different squares for balancing carryover effects. Both squares had 4 steers and squares were conducted simultaneously. Each experimental period consisted of 11 d of adaptation to diets and 4 d of experimental measurements. The ruminal cannulas measured 7.5 cm center diameter (Bar Diamond, Parma, ID). Steers were housed in individual dry lot pens and offered the experimental diets twice a day at 0700, 1800 h for 90% of intake to allow no refusal. The fibrolytic enzyme preparation containing xylanase and cellulase activities (Fibrozyme, Alltech Inc., Nicholasville, KY, USA). The treatments were: T1) *B. mulato* and *B. decumbens* of 28 d with enzyme; T2) *B. mulato* and *B. decumbens* of 28 d without enzyme; T3) *B. mulato* and *B. decumbens* of 56 d with enzyme; T4) *B. mulato* and *B. decumbens* of 56 d without enzyme. Nylon bags were incubated with samples of hay with a size of 2 mm in the rumen at 0, 12, 24, 48 and 96 h and the DM and OM remaining at each incubation time was fitted to the nonlinear regression model using NLIN procedure of SAS and the pH was measured from the liquid ruminal to 0, 3, 6, 9 and 12 h were analyzed using MIXED procedure of SAS. The in situ digestibility of dry matter and ruminal pH, was not change by the application of the compound enzymatic exogenous ($P \geq 0.05$). We can conclude that the use of this enzymes do not affect the digestion of the dry matter of the hay of *B. mulato* and *B. decumbens* cut at 28 and 56 d.

**Table 1.** In situ digestibility of DM for the experimental materials

| | T1[1a] | T1 | T2[b] | T2 | T3[2a] | T3 | T4[b] | T4 |
|---|---|---|---|---|---|---|---|---|
| Component | EZ+ | EZ- | EZ+ | EZ- | EZ+ | EZ- | EZ+ | EZ- |
| In situ digestibility | | | | | | | | |
| 96 | 69.73 | 73.73 | 66.62 | 66.50 | 72.69 | 72.13 | 61.72 | 62.52 |
| 48 | 68.79 | 69.39 | 61.07 | 60.66 | 65.98 | 68.28 | 55.40 | 54.65 |
| 24 | 57.18 | 57.55 | 49.58 | 50.09 | 57.66 | 57.74 | 44.59 | 43.45 |
| 12 | 44.87 | 44.22 | 37.81 | 38.65 | 43.77 | 48.02 | 33.51 | 33.24 |
| 0 | 29.68 | 29.91 | 25.57 | 25.55 | 28.44 | 28.99 | 20.14 | 21.79 |

[1]*Brachiara mulato* with or without enzyme. [a] 28 or [b] 56 d.
[2]*B. decumbens* with or without enzyme.

**Key words:** *Brachiaria*, enzymes, digestibility

**T358 Method evaluation for determining digestibility of rumen undegraded amino acids in blood meal.** S. E. Boucher*[1], S. Calsamiglia[2], M. D. Stern[3], C. M. Parsons[4], H. H. Stein[4], C. G. Schwab[5], K. W. Cotanch[6], J. W. Darrah[6], and J. K. Bernard[7], *[1]Kemin AgriFoods North America Inc., Des Moines, IA, [2]Universitat Autònoma de Barcelona, Bellaterra, Spain, [3]University of Minnesota, St. Paul, [4]University of Illinois, Urbana, [5]Schwab Consulting LLC, Boscobel, WI, [6]William H. Miner Agricultural Research Institute, Chazy, NY, [7]University of Georgia, Tifton.*

To evaluate various methods for estimating digestibility of rumen undegraded AA in blood meal (BM), 5 BM samples (2 bovine, 3 porcine) were obtained. One bovine sample was heated at 125°C for 2 h to generate an additional bovine sample and one porcine sample was heated at 110°C for 2 h (n = 6). Samples were ruminally incubated in situ for 16 h in 3 lactating cows fed a 55% forage diet. Rumen undegraded residues (RUR) were pooled by sample and analyzed for AA. Digestibility of AA in the RUR was determined via the mobile bag technique (MBT) in dairy cows, precision fed cecectomized rooster assay (CRA), modified 3-step procedure (MTSP), and homoarginine assay (HA; estimates available Lys). For the MBT, 0.8 g of each RUR was weighed into 24 polyester bags, soaked in a pepsin/HCl solution for 2 h, and introduced into 2 duodenally cannulated cows. Bags were collected from the feces and undigested residues were analyzed for AA. Digestibility of AA was calculated by disappearance. To calculate standardized AA digestibility using the CRA, RUR were tube fed to 4 birds per sample, and total excreta collected for 48 h and analyzed for AA. For the MTSP, 5 g of each RUR were weighed into 2 polyester bags and incubated (38°C) sequentially in a pepsin/HCl solution for 1 h then a pancreatic solution for 24 h in Daisy$^{II}$ incubator bottles. Digestibility of AA was calculated by disappearance. For the HA method, RUR were guanidinated and analyzed for Lys and HA content. Percent Lys converted to HA was calculated. The REG procedure of SAS was used for data analysis. $R^2$ values for Lys digestibility using MTSP, CRA, and HA procedures compared with the MBT in cows (independent variable) were 0.89, 0.62, and 0.05, respectively, and the $R^2$ values for total essential AA (EAA) digestibility using MTSP and CRA compared with MBT were 0.89 and 0.92, respectively. Using MBT in dairy cows as a reference, it appears that HA method is not a good approach to determine available Lys in BM, CRA was adequate to determine digestibility of total EAA in BM, and MTSP is a good approach to estimate digestibility of both Lys and total EAA in BM.

**Key words:** blood meal, rumen-undegraded protein, mobile bag technique

**T359 In vitro modification of ruminal and post ruminal metabolism by lignosulfonate and polysaccharide protected microminerals.** M. Ruiz-Moreno*[1], E. Seitz[1], M. D. Stern[1], and J. Garrett[2], *[1]University of Minnesota, St. Paul, [2]Quali Tech Inc., Chaska, MN.*

Ruminal and postruminal availability of trace minerals is affected by chemical nature and presence of chelating agents such as lignin derived phenolic compounds. The aim of this study was to evaluate effects of lignosulfonate and polysaccharide-protected minerals on in vitro rumen fermentation, ruminal and post ruminal partition of Cu, Zn and Mn. Eight dual flow continuous culture fermenters were used during 2 consecutive 10-d periods in a 2 × 2 factorial arrangement of treatments. A synthetic diet consisting of 38% cellulose, 34% starch, 20% powdered whey, 5.3% vegetable oil and 2.5% sugar provided substrate for microbial metabolism. Sulfur was added as $NaSO_4$ or S-bound lignosulfonate to a final concentration of 0.75% of DM. Lignosulfonate was added at 0 (LIG0) or 5% (LIG5) of DM. Copper, Zn and Mn were added as $CuSO_4$, $ZnSO_4$ and $MnSO_4$ or as polysaccharide protected Cu, Zn and Mn (SQM protected minerals, Quali Tech Inc.; SQM- or SQM+, respectively) to a final concentration of 16, 56 and 71 ppm of DM, respectively. At the end of each period, solid and liquid fractions from fermenters outflows were subjected to pepsin-pancreatin enzymatic digestion. Apparent and true OMD (%) were not affected by treatments ($P > 0.05$). Addition of LIG5 decreased ($P < 0.05$) daily flow of non $NH_3$-N, efficiency of microbial protein synthesis, total VFA and molar proportion of acetic acid, but increased ($P < 0.05$) propionic, valeric and caproic acid while SQM+ decreased molar proportion of propionic acid. Addition of LIG5 increased ($P < 0.05$) ruminally soluble Cu and Mn, while SQM+ reduced ruminally soluble Cu. Concentration of bacterial Cu and Zn increased with SQM+ in absence of lignosulfonate ($P < 0.05$). Addition of LIG5 resulted in higher enzymatic release of Zn from solids outflow but lower from bacterial pellets ($P < 0.05$). Mean, minimum and maximum fermentation pH were higher ($P < 0.05$) with LIG5. Addition of lignosulfonate induced major changes in ruminal fermentation. Protected minerals decreased rumen soluble Cu and increased bacterial Cu and Zn without affecting predicted post ruminal release of minerals.

**Key words:** protected minerals, rumen, lignosulfonate

**T360 Factors affecting estimation of spoilage indices in silage 2: Effects of amount of silage evaluated and type of container.** N. Cavalcanti[1,2], J. Leite[1,2], L. G. Paranhos*[1], O. C. M. Queiroz[1], K. G. Arriola[1], and A. T. Adesogan[1], *[1]University of Florida, Gainesville, [2]Federal University of Pernambuco, Recife, Pernambuco, Brazil.*

Aerobic stability is a measure of the shelf life of silage and an indirect measure of the likelihood of undesirable microbial activity, which predisposes to heating, nutrient depletion and growth of pathogenic organisms. Different methods are used for this assay and this likely affects the outcome. This project aimed to examine effects of container type and amount of silage evaluated on the aerobic stability of corn silage. Three different amounts of corn silage, 1, 2, or 3 kg were packed at the same density (550 kg/m3) into 20 L plastic buckets (PB) or 20 L styrofoam containers (SC) in quadruplicate. Wireless temperature sensors were placed in the center of the silage mass in each container and set to record temperatures every 30 min for 14 d. Ambient temperature was similarly measured. Aerobic stability was estimated as the time (h) before silage and ambient temperature differed by more than 2°C. Maximum and minimum temperatures and an instability index estimated as the area under the temperature curve during the aerobic exposure period were recorded. The experiment had a ran-

domized complete block design and a 2 (container type) × 3 (amount of silage) factorial treatment arrangement. The statistical model contained silage amount and container effects and the interaction. Minimum temperature was greatest for 1 kg forage in SC and for 3 kg silage in PB (amount x container interaction, $P = 0.02$). Using SC resulted in greater aerobic stability (168.9 vs.79.9 h; $P < 0.01$) and greater minimum temperatures (2°C difference) compared with using PB. Using 3 kg of silage resulted in greater maximum temperature (37.4 vs. 29.0; $P < 0.01$), greater temperature range (20.3 vs. 11.2°C; $P < 0.01$), and greater area under the temperature curve compared with using 1 kg ($P < 0.01$). These data showed that container type and amount of silage evaluated influence the aerobic stability result.

**Key words:** aerobic stability, corn silage, methodology

**T361 Infusion of marker solution into intact digesta for measurement of the ruminal clearance of volatile fatty acids.** J. C. de Resende Júnior*, J. L. P. Daniel, F. da C. Meireles, M. B. Moreira, and R. F. de Lima, *Universidade Federal de Lavras.*

The removal (clearance) of volatile fatty acids (VFA) of the reticulorumen occurs by absorption through the wall or passage to the omasum. This study aimed to validate a new technique for infusion of marker solution into intact ruminal digesta comparing with another technique which has known efficiency for measurements of the ruminal clearance of VFA. Four cows were allocated to 4 treatments in split plot design, aligned in a 2 × 2 factorial arrangement which was diet and method of infusion of markers applied concurrently in 2 periods of 18 d. The 4 combinations were: forage diet and infusion of markers into intact (ID) or evacuated digesta (ED); forage plus concentrate and infusion of markers into intact or evacuated digesta. Four liters of markers solution containing Cr-EDTA associated with valeric acid were added to the ruminal digesta. Rumen fluid samples were serially collected and analyzed for pH, VFA concentration and Cr. The fractional clearance rate of total VFA was estimated by the exponential decay rate of the valerate concentration over time. The clearance of VFA by passage to the omasum was assumed to be equivalent to the decay in ruminal Cr concentration and the fractional clearance rate of absorption was estimated by difference. The fractional rates of total clearance (ID = 37.8%/h; ED = 30.5%/h) and absorption (ID = 26.0%/h; ED = 21.1%/h) of VFA did not differ between techniques ($P = 0.30$ and $P = 0.52$, respectively), demonstrating that the infusion technique into ID is comparable to the infusion technique into ED. The fractional rate of passage of the fluid phase (ID = 11.8%/h; ED = 9.4%/h), however, was lower ($P = 0.06$) when the marker solution was added into the evacuated digesta, probably reflecting the destabilization of the rumen environment during the evacuation and the largest volume of fluid observed in animals with evacuated digesta. It is concluded that the infusion of marker solution into intact digesta with homogenization performed by ruminal motility is effective and seems to be the better choice for the VFA ruminal clearance determination because it allows measurements under more normal physiological conditions.

**Key words:** acidosis, measurement of metabolizable energy, ruminant stomach

**T362 Adjustment of in vitro rumen fermentation protocol for testing products based on rumen pH regulation and the impact of Acid Buf.** S. Taylor*[1], E. Pennala[2], and J. Apajalahti[2], *[1]Celtic Sea Minerals Ltd., Cork, Ireland, [2]Alimetrics Ltd., Espoo, Finland.*

Investigating the mode of action of buffering materials by in vitro rumen fermentation is restricted because protocols normally use strong buffers to compensate for the lack of acid absorption from the system. Optimization of buffer strength and volume, and the amount / type of feed enables the mimicking of acidosis and the testing of products designed to impact on this challenge. Simulation protocol: The simulation used 1 g (DM) feed composed of 50% grass silage, 40% barley meal and 10% soy. The buffer based on bicarbonate and phosphate (Agriculture Handbook, Vol 379, USDA 1970) was diluted with 0.9% NaCl as indicated below. The study with 12 replicates was inoculated with 5% of fresh, strained rumen fluid from a cow on a high energy diet and continued for 12 hours at 37°C. Anaerobic techniques were applied throughout. The treatments were: undiluted buffer and this diluted to 1:2 and 1:4 in constant volume (40 ml) A treatment with 1:4 diluted buffer + 50 mg of Acid Buf/40 ml was included. Total gas production, pH, and short-chain fatty acids (SCFAs) were determined at various time points. Cumulative methane production and bacteria were analyzed at the end. SCFAs and methane were analyzed by GC and bacteria by flow cytometry. Dilution of the buffer allowed acidity to increase, which led to suppression of bacterial growth and metabolism. Addition of Acid Buf in the fermentation with the 1:4 diluted buffer significantly reduced the drop of pH and maintained higher bacterial activity. Methane to acid ratio (ml/mM) was lower than with the weaker buffering

**Table 1.** Results

| | Buffer 1:1 | Buffer 1:2 | Buffer 1:4 | Buffer 1:4 + Acid Buf |
|---|---|---|---|---|
| Gas production at 12 h (mL) | 102[a] | 86[b] | 44[d] | 60[c] |
| pH, 4 h | 6.83[a] | 6.42[b] | 6.00[c] | 6.10[c] |
| pH, 12 h | 6.73[a] | 6.15[b] | 5.31[c] | 5.69[b] |
| SCFA, 12 h (m*M*) | 68[a] | 67[a] | 52[b] | 65[a] |
| Acetate, 12 h (m*M*) | 29[a] | 24[b] | 16[d] | 21[c] |
| Propionate, 12 h (m*M*) | 20[a] | 19[a] | 13[c] | 17[b] |
| Methane 12 h (mL) | 4.3[a] | 4.1[a] | 1.9[c] | 2.9[b] |
| CH4/SCFA (mL/m*M*) | 6.3% | 6.1% | 3.6% | 4.5% |
| Bacteria (cells/mL) | 8.9E+0.9[a] | 6.1E+09[b] | 3.3E+09[d] | 4.5E+09[c] |

Numbers with the same superscript are not significantly different ($P = 0.05$).

**Key words:** acidity, rumen, simulation

**T363 Impact of different sources of hydrolysable and condensed tannins on rumen fermentation and methane production in vitro.** F. Hassanat* and C. Benchaar, *Agriculture and Agri-Food Canada, Dairy and Swine Research and Development Centre, Sherbrooke, Qc, Canada.*

Tannins added to animal diet may generate positive impact on energy and protein utilization in the rumen. The objective of this study was to examine the impact of different sources and levels of condensed and hydrolyzable tannins on rumen microbial fermentation in vitro (24-h batch cultures). Condensed tannin extracts from Acacia (AT; *Acacia mearnsii*; 82% DM) and Quebracho (QT; *Schinopsis balansae*; 90% DM) and hydrolysable tannin extracts from Chestnut (ChT; *Castanea sativa*; 75% DM) and Valonia (VT; *Quercia vallonea*; 71% DM) were used. In vitro incubations (repeated 4 times) were conducted in a completely randomized block design using a control (CTL; 0%) and each source of tannin at 2, 5, 10, 15 and 20% of total mixed ration DM. Each

treatment was tested in quadruplicate. Differences between treatments and CTL were declared significant at $P \leq 0.05$ using Dunnett's comparison test. Gas production (GP) was reduced at ≥5% of AT, while at 2% or more, QT produced less gas than CTL. At ≥5% concentration, both AT and QT reduced total volatile fatty acids (VFA) and $CH_4$ concentrations. No effect was observed on acetate ($C_2$) proportion at any level of AT or QT while propionate ($C_3$) proportion was slightly increased at ≥ 10% of AT or QT, resulting in lower $C_2$:$C_3$ ratio. Addition of ChT at ≥5% reduced GP, $CH_4$ and VFA concentrations compared with CTL. Proportion of $C_2$ increased when ChT was supplied at ≥10% while no effect of ChT was observed on $C_3$ proportion or $C_2$:$C_3$ ratio. Supplying VT at ≥2% reduced GP while a level of VT ≥5% was required to decrease $CH_4$ concentration. Total VFA concentration was reduced only at ≥10% of VT while $C_2$ proportion was increased at ≥2% VT and no change was noted for $C_3$ and $C_2$:$C_3$ ratio. Proportions of isovaleric, and valeric and ammonia concentration were decreased at all levels of tannin sources added, indicating reduced protein degradation. At low concentrations (2–5%), tannins have the potential to reduce $CH_4$ production and ruminal protein degradation without deleterious effects on fermentation.

**Key words:** in vitro fermentation, methane, tannins

**T364 Changes in ruminal bacterial community composition following feeding of silage inoculated with a commercial silage inoculant.** R. Mohammed*[1,2], D. M. Stevenson[1], K. A. Beauchemin[2], P. J. Weimer[1], and R. E. Muck[1], *[1]USDA-ARS, Madison, WI, [2]AAFC, Lethbridge, AB, Canada.*

Some silage inoculants yield an increase in milk production, possibly through altering the rumen microflora. We hypothesized that alfalfa silage treated with a commercial inoculant (*Lactobacillus plantarum*, LP) would alter rumen bacterial community composition (BCC) compared with silage without inoculant (Ctrl). Eight rumen-cannulated Holstein cows were allotted to 2 diets (Ctrl- or LP-treated silage) in a double crossover design with 4 28-d periods. Diets were formulated to contain (per kg DM) 280 g NDF and 162 g CP, and contained (g/kg DM): alfalfa silage, 509; corn silage, 206; high-moisture shelled corn, 214, soy hulls, 47; plus minerals and vitamins. Ruminal digesta were collected just before feeding on the last 3 d of each period, and were separated into solid and liquid phases. Microbial DNA was extracted from each phase, amplified by polymerase chain reaction (PCR) using domain-level bacterial primers, and subjected to automated ribosomal intergenic spacer analysis (ARISA) for comparison of BCC. Correspondence analysis of the 266 peaks in the ARISA profile across the 192 samples revealed that the first 2 components contributed 6.8% and 4.2% to the total variation in the profile. Data points representing the liquid and solid phases clustered separately, indicating that these phases differed in BCC. Treatment effects were not apparent from the ARISA profiles. However, the relative population size (RPS) of LP, determined by quantitative PCR, was greater in treated silage compared with the Ctrl ($P < 0.01$). Data points corresponding to certain individual cows clustered separately, and the most distinctive bacterial communities were those associated with milk fat-depressed cows. The RPS of one bacterial species, *Megasphaera elsdenii*, was greater in fat-depressed cows. However, mean RPS of *M. elsdenii* did not differ between the treatments. The results indicate that a silage inoculant can affect rumen bacterial composition beyond elevating the population of the specific microbial inoculant.

**Key words:** rumen, silage inoculant, microbial populations

**T365 Effect of a dietary antioxidant with different substrate on rumen fermentation in vitro.** Y. Wang*[1,2], J. Wang[1], M. Vazquez-Anon[2], H. Cao[2], G. Zanton[2], and J. Liu[1], *[1]Institute of Dairy Science, Zhejiang University, Hangzhou, P. R. China, [2]Novus International Inc., St. Louis, MO.*

The objective of the study was to evaluate the effect of a dietary antioxidant (AOX; AGRADO® Plus, Novus International) on rumen fermentation with different dietary ingredients as substrate in vitro, in the absence or presence of 500 mg/kg AOX. Data were analyzed as a completely randomized design using the MIXED procedure. Neither substrate nor AOX had significant effect on rumen pH. Inclusion of different substrates significantly affected gas production, organic matter digestibility, and total VFA ($P < 0.05$), where corn appeared to have highest values, while cotton seed had lowest ones, compared with extruded soybean and DDGS. Extruded soybean had higher $NH_3$-N than corn ($P < 0.05$), and cottonseed and DDGS were intermediate in $NH_3$-N levels. AOX had no significant effect on gas production, organic matter digestibility, $NH_3$-N, or total VFA production. However, addition of AOX significantly increased the molar proportion of propionate ($P < 0.05$), and tended to decrease the molar proportion of acetate ($P = 0.10$). Different substrates had similar anti-oxidative status in the rumen ($P > 0.05$). AOX significantly increased the total antioxidant capacity ($P < 0.01$), but did not change other antioxidant biomarkers (superoxide dismutase, malondialdehyde, glutathione peroxidase, and hydrogen peroxide). Except for *Fibrobacter succinogenes*, the population of *Ruminococcus flavefaciens*, *Ruminococcus albus*, fungi, protozoa and *Butyrivibrio fibrisolvens* were significantly affected by substrate treatment ($P < 0.01$). Addition of AOX increased *Ruminococcus albus* population ($P < 0.05$). There was no significant interaction between substrate and AOX for fermentation patterns, oxidative status or rumen microflora. It is concluded that different substrates significantly affected the fermentation pattern and microflora, but not for anti-oxidative status in the rumen. Addition of AOX improved the total anti-oxidative status, increased the molar proportion of propionate and *Ruminococcus albus* population, regardless of substrate type.

**Key words:** antioxidant, anti-oxidative status, rumen fermentation

**T366 Effect of dietary roughage and sulfur concentration on hydrogen sulfide production from corn-based diets containing dried distillers grains.** E. Seitz*, A. Carpenter, M. Ruiz-Moreno, M. D. Stern, and G. I. Crawford, *University of Minnesota, St. Paul.*

An in vitro rumen fluid incubation was conducted using differing dietary concentrations of roughage (R) and sulfur (S) in a 3 × 2 + 2 factorial arrangement of treatments during 4 consecutive 24-h periods. Isonitrogenous dietary treatments included a corn-based control diet with no distillers grains (DG), 9% R, and 0.18% S (CON); a high R treatment with 27% R, 40% DG and 0.50% S (HRHS), and 6 treatments arranged in a 3 × 2 factorial with 3 S concentrations (0.3, 0.4, and 0.5%; LS, MS, HS, respectively), and 2 R concentrations (3 and 9%; LR and MR, respectively). Grass hay served as the roughage source and S concentrations were achieved through combination of 2 DG with differing S concentrations. Rumen fluid adapted to each treatment was mixed with saliva in a 1:1 ratio (10 mL each) and incubated with 0.2 g substrate DM in crimp-sealed, 50 mL serum bottles (n = 24; 3 reps/trt) at 39°C. At 5 and 24-h post-incubation, gas production was measured and a subsample of headspace gas was analyzed for hydrogen sulfide ($H_2S$). Final pH was measured at the end of each 24-h incubation. These results indicate that DG inclusion generally increased

batch culture pH, and compared with CON, the MS and HS treatments had higher total μg $H_2S$ and μg $H_2S$/mL gas.

**Table 1.** Effect of dietary roughage and sulfur concentration

| Parameter | CON | LRLS | LRMS | LRHS | MRLS | MRMS | MRHS | HRHS | *P*-value |
|---|---|---|---|---|---|---|---|---|---|
| Final pH | 5.44[a] | 5.55[ab] | 5.65[be] | 5.62[bc] | 5.67[ce] | 5.66[be] | 5.81[d] | 5.95[f] | <0.0001 |
| Total $H_2S$ (μg) | 28.3[a] | 51.1[ad] | 81.9[bd] | 77.9[bd] | 57.2[acd] | 89.4[bd] | 113.3[b] | 93.1[bc] | 0.01 |
| Total gas (mL) | 38.9[a] | 35.9[bd] | 35.0[bc] | 37.8[ad] | 35.8[bd] | 37.1[acd] | 37.0[acd] | 33.4[b] | 0.01 |
| μg $H_2S$/mL gas | 0.7[a] | 1.4[ac] | 2.3[bc] | 2.1[bc] | 1.6[ac] | 2.4[bc] | 3.0[b] | 2.8[b] | 0.005 |
| $NH_3$-N (mg/100 mL) | 4.2 | 3.9 | 3.8 | 5.1 | 3.8 | 4.5 | 4.7 | 5.1 | 0.52 |

[abcdef]Means in the same row with uncommon superscripts differ ($P < 0.05$).

**Key words:** hydrogen sulfide, in vitro, rumen

**T367 Effects of hops on rumen fermentation and bacterial populations using the rumen simulation technique.** N. Narvaez*[1], Y. Wang[1], Z. Xu[1], T. Alexander[1], S. Garden[2], and T. McAllister[1], *[1]Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada, [2]John I. Haas Inc., Washington DC.*

A rumen simulation technique (Rusitec) experiment was conducted to assess the effects of supplementation of 3 varieties of hops on rumen fermentation and rumen bacterial communities. The treatments were Control (no hops) and 3 hop varieties (Cascade, CAS; Millenium, MIL and Teamaker, TM). Two RUSITEC with 8 vessels each were used. Each vessel was initially inoculated with rumen solids and liquids from cattle fed a barley silage-barley grain diet and fermenters were fed 10 g of a barley silage-barley grain diet daily. Hops extract (800 μg/mL) was added so as to have 2 replicate fermenters per variety. Microbial protein synthesis (MN) was estimated using $^{15}N$ labeled ammonium sulfate and principal ruminal bacteria were quantified using real-time polymerase chain reaction (qPCR). Addition of all hop varieties reduced ($P < 0.001$) total gas and $CH_4$ production per g of truly digested dry matter (TDDM). True DM disappearance was reduced ($P < 0.05$) by CAS and MIL but only MIL reduced ($P < 0.001$) neutral detergent fiber disappearance (NDFD). Productions of volatile fatty acids (VFA) and MN were unaffected by hops, but the acetate:propionate (A:P) ratio was decreased ($P < 0.001$) with all hop varieties. Proportions of 16S rDNA gene of *F. succinogenes* and *S. bovis* were decreased ($P < 0.05$) with addition of MIL and TM whereas *S. bryantii* was increased ($P < 0.001$) by CAS. The proportion of *Archae* marker gene in solid fraction was also reduced ($P < 0.05$) by all 3 hops. The decreased methane production by hops is likely due to their effects on altering rumen microbial community by reducing methanogens and cellulolytic bacteria and reducing A:P ratio of the VFA. Inclusion of hops in ruminant diet may have potential to reduce methane production and thereby improve feed efficiency.

**Key words:** hops, rumen bacteria, rumen fermentation

**T368 Effect of nitrate, sulfate, monensin, and corn gluten feed on in vitro ruminal methane production.** C. Davis[1], S. Ghimire*[1], T. Wiles[1], Z. Wen[2], M. A. McCann[3], and M. D. Hanigan[1], *[1]Department of Dairy Science, Virginia Polytechnic Institute and State University, Blacksburg, [2]Department of Biological Systems Engineering, Virginia Polytechnic Institute and State University, Blacksburg, [3]Department of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg.*

Two in vitro studies were conducted using hay and total mixed dairy ration (TMR) as substrates to evaluate the effect of nitrate and Monensin on methane production. Study 1 was conducted to compare the effectiveness of different doses of nitrate and Monensin on methane production, and study 2 tested the effects nitrate, sulfate, and corn gluten feed (CGF). Four levels of nitrate (0, 2.5, 5, and 7.5% of DM) and 2 doses of Monensin (0, and 4 mM) were tested for each diet in study 1. Two doses of nitrate, 2 doses of sulfate (0 or 4% of DM each), and the absence or presence of CGF in the diet (22% of DM) were tested in study 2. In study 1, nitrate was added without lowering the TMR protein level. Treatments were isonitrogenous in study 2. Each study was replicated 3 times and contained one bottle for each treatment to measure methane production and a second bottle to measure total gas production. Bottles were inoculated with 20 mL of strained rumen fluid plus 60 mL of McDougall's buffer. Ruminal fluid was collected from 2 nonlactating, Holstein cows, one on hay and the other on a lactating cow ration. Hay substrate was used for the hay diets and TMR for the TMR diets. Bottles were incubated for 48 h at 39°C. Total gas production was measured at different time intervals after incubation by water displacement and methane production was measured by displacement after removal of CO2 using sodium hydroxide. Cumulative methane production was greater ($P < 0.05$) for the TMR diet. Monensin, sulfate, and CGF did not have significant effects on methane production ($P > 0.05$). Nitrate reduced methane production in both studies. In study 1, the reduction as compared with the controls was 16.92%, 28.03%, and 30.43% for hay and 29.41%, 35.95% and 41.53% for TMR at concentrations of 2.5, 5, and 7.5% of DM, respectively. In study 2, nitrate reduced methane production by 25.74% for hay and 13.23% for TMR. Total gas production was reduced when nitrate was present ($P < 0.05$) in study 1, but the reduction was not significant in study 2. These results suggest that nitrate can be used as a strategy to reduce methane production in cattle.

**Key words:** dairy cow, ruminal methane, nitrate

**T369 Effects of microwave irradiation on ruminal dry matter degradability of canola and corn gluten meal.** M. Dehghan-Banadaky[1], H. Khalilvandi-Behroozyar*[1,2], H. R. Khazanehi[3], and N. Vahdani[1], *[1]Department of Animal Science, University of Tehran, Karaj, Tehran, Iran, [2]Department of Animal Science, University of Urmia, Urmia, West Azerbaijan, Iran, [3]Department of Animal Science, University of Manitoba, Manitoba, Canada.*

Microwave energy causes a rise in the temperature within a penetrated medium as a result of rapid changes of the electromagnetic field. This study was conducted to evaluate effects of 900 W microwave irradiation for 4 and 6 min on dry matter degradability of canola and corn gluten meals (CGM) using nylon bag technique. The DM of meals was determined by oven drying of a 1 g sample in triplicate. Based upon this value, sufficient water was added to increase the moisture content of 2 kg of canola meal to 250 g/kg. Two samples (each of 500 g) were subjected to microwave irradiation at a power of 900 W for 4 and 6 min. Dry matter degradability was determined using 3 ruminally fistulated non lactating Holstein cows, fed balanced rations with forage:concentrate ratio of 60:40. Samples were ground to pass 2 mm screen and 5 g was weighted into nylon bags with 50 micron pore size (sample size:surface area was 12.5 mg/cm$^2$). Duplicates were

incubated for 2,4,8,12,24 and 48 in ventral rumen. Effective degradability (ED) was calculated with NEWAY computer package. CRD design, GLM PROC of SAS 9.1 and Duncan test option was used for data analysis. In the case of canola meal results (Table 1) showed that microwave irradiation decreased rapidly degradable fraction and increased lag time ($P \leq 0.05$). Also, a trend ($P \leq 0.08$) was observed for increasing of potentially degradable fraction and reduction of the rate of degradation of b fraction with irradiation. Increasing the irradiation time, decreased effective degradability of dry matter ($P \leq 0.08$). Microwave irradiation resulted in statistically significant ($P \leq 0.05$) increase in DM ED of CGM in rumen outflow rates of 0.05 and 0.08 $h^{-1}$. Although treatments were resulted in increased b and reduction of a fraction ($P \leq 0.1$), degradation rate of b fraction also increased ($P \leq 0.12$). Previous reports about heat treatment of CGM revealed that ruminal effective degradability decreased with heat treatment, but our results showed increased DM degradability with irradiation. Further studies about effects of microwave irradiation on ruminal nutrient degradability of CGM, recommended.

**Table 1.** Rumen DM degradation parameters of untreated and microwave irradiated canola meal

| | Control | 4 min | 6 min | SEM |
|---|---|---|---|---|
| a (percentage) | 27.73[a] | 26.59[b] | 24.81[c] | 0.048 |
| b (percentage) | 60.00 | 61.33 | 62.40 | 0.511 |
| c ($h^{-1}$) | 0.12 | 0.11 | 0.09 | 0.004 |
| Lag time (h) | 1.10[b] | 1.68[a] | 1.70[a] | 0.085 |
| ED (percentage, K = 0.02) | 77.37 | 76.67 | 74.27 | 0.559 |
| ED (percentage, K = 0.05) | 66.60 | 65.33 | 61.97 | 0.999 |
| ED (percentage, K = 0.08) | 59.17 | 57.63 | 54.07 | 1.145 |

Means within each row with different superscripts are significantly different ($P \leq 0.05$).

**Key words:** microwave irradiation, protein concentrate, dm degradability

**T370 Evaluation of two protein hydrolyzates as a source of soluble protein to foster ruminal microbial growth.** A. Aris[1], A. Serrano[1], F. Fabregas[1], J. Polo[3], C. Rodriguez[3], and A. Bach*[1,2], *[1]Ruminant Production, Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Caldes de Montbui, Barcelona, Spain, [2]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain, [3]APC EUROPE, S.A. R&D department, Granollers, Barcelona, Spain.*

The aim of this study was to determine whether the protein hydrolyzates AproCel and Pepton (APC Europe, Barcelona, Spain) would stimulate rumen microbial growth in comparison to Tryptone, which is the gold-standard of supplementary N for microbes. Ruminal samples from 3 different animals were incubated for 12 h following the Tilley-Terry procedure. There were 4 treatments applied to the ruminal samples: Negative control (CTR), with no N supplement added, positive control Tryptone added at the rate of 2% (TRY) and Pepton (PEP) and AproCel (APR) added at a rate (2.10 and 2.24%, respectively) providing the same amount of N as Tryptone 2%. After incubation, a liquid sample was obtained to determine total volatile fatty acids (VFA) concentrations, pH, and microbial growth (determined by quantitative real time PCR). Data were analyzed with an ANOVA using the treatment as the main effect. Total VFA production was numerically greater in all tubes with N supplementation in comparison to CTR (56.3mM ± 12.24), with this difference being significant ($P < 0.05$) for APR (100.7mM ± 12.24) and Tryptone (128.9mM ± 12.24). Pepton supplementation resulted in an intermediate VFA concentration. Ruminal fluid experienced in all cases a slight increase in pH compared with CTR (6.96 ± 0.03). Pepton (7.20 ± 0.03) showed the greatest increase of pH ($P < 0.05$). The increase in pH indicates that microorganisms used part of the N supplements as a source of energy leading to an excretion of $NH_3$. Probably the production of $NH_3$ was lowest with PEP, because this treatment resulted numerically in the least VFA production among the 3 protein supplements, and thus pH would be expected to be greater even in the absence of $NH_3$. Gram-positive bacteria grew equally among the 3 treatments, whereas gram-negative growth was highly stimulated ($P < 0.05$) by PEP (PEP: 4.52 ± 0.6 ratio to control vs CTR: 1.92 ± 0.6 ratio to control). In conclusion, both APR and PEP, are readily available N sources for microbial protein synthesis. In addition, PEP stimulates growth of gram-negative bacteria to a greater extent than TRY and APR.

**Key words:** microbial growth, protein hydrolyzate, supplementary nitrogen

**T371 Effects of protein protection with orthophosphoric or malic acid and heat in lamb fattening diets.** F. Díaz-Royón*, J. M. Arroyo, M. R. Alvir, V. Jimeno, S. Sanchez, and J. González, *University of Politécnica de Madrid, Madrid Spain.*

The objective of this study was to evaluate the efficiency of using acid-heat treated (121°C, 1 h, plus residual oven heat) sunflower and pea meals on diets fed to fattening lambs. Ninety "Entrefino" cross male lambs from three commercial farms (average initial body weight = 14.6; 15.3, and 13.3 kg) were randomly assigned to five diets with different levels of protein and acid treatment, and fattened to an average body weight at slaughter of 25 kg. The control diet (C; CP =18%) contained conventional soybean, sunflower and pea meals. In three of the treatment diets, orthophosphoric acid-protected meals (TM) replaced conventional sunflower and pea meals (CF; CP=18%) and soybean meal was progressively remove (SMF; CP=16.7% and STF; CP= 15.6%). In the last diet (SMM; CP= 16.7%) malic acid substituted ortophosphoric acid. Wheat straw (roughage source) and concentrate were offered. Eighteen lambs, 3 animals per pen, allocated to 6 pens, were assigned to each diet. Data were analyzed using a factorial analysis with initial body weight as covariate and farm of origin as block. Treatments were compared through the following contrasts: C. vs. CF, SMF, STF; CF vs. SMF, STF; SMF vs. STF; C. vs. SMM; SMF vs. SMM). Average daily gain (ADG), carcass yield (CY), dorsal fat (DF) and kidney pelvic fat (RPF) were analyzed by animal. Intake and feed conversion (FC), were analyzed by pen. There was no diet effect on any parameter observed which suggests that when protected proteins are used, it is possible to work with 15.6% CP (DM basis) reducing the need to include vegetable protein meals. Lambs on SMM had higher ADG (15.2%; P= 0.042), and better CY (1.3%; P= 0.037) than on SMF. Improved efficiency can be attributed to greater protection by malic acid (Arroyo, 2007) or a higher propionic acid production as result of a shift in rumen fermentation in response to malic acid inclusion.

**Key words:** acid-heat treatment, fattening lamb, protein protection

**T372 Identification of several novel fungal species in feed samples from the southeast United States.** J. D. Chapman*[2], Y. Q. Wang[1], and N. E. Forsberg[1], *[1]OmniGen Research, Corvallis, OR, [2]Prince Agri Products, Quincy, IL.*

Fungi grow freely on preserved feeds. Concerns about the presence of fungi include their production of mycotoxins, their invasive (mycotic)

potential and their metabolism of nutrients. Despite years of work in this area, the full spectrum of fungi which grow on silages and the implications of their growth have not been fully established. The goal of this study was to identify unknown fungi found growing on a balage sample in Georgia and on a corn silage sample in Florida. Samples of fungi-contaminated feeds were recovered and inoculated onto Sabouraud culture plates. Pure cultures were selected from the plates and DNA extracted from each. The ITS-1 domain was amplified by polymerase chain reaction (PCR) using pan-fungal primer sequences. PCR products were electrophoresed on agarose and the fragments corresponding to the ITS-1 fragment were excised, purified then sequenced. Four fungi were identified of which 3 were relatively unknown. The 4 included *Aspergillus clavatus* (a dark green-black fungus), *Coccidioides immitis* (a pale blue fungus), *Gibberella zeae* (also known as *Fusarium graminearum*; a red fungus), and *Neosartorya fischeri* (a white fungus). Based on the published abilities of these species to secrete mycotoxins and/or to cause invasive mycosis, each holds potential to adversely affect animal health. *A. clavatus* secretes a broad spectrum of mycotoxins including alanyltryptophan, cytochalasin E, kotanin, nortryptoquivaline, and patulin. *C. immitis* is a Level-III pathogenic fungus, resides principally in the Southern US and is responsible for Valley Fever. Reports of mammary *C. immitis* infections in dairy cattle exist. *G. zeae* secretes a variety of mycotoxins including deoxynivalenol, an important immunosuppressive toxin. Finally, *N. fischeri* secretes aflatoxin, fumetrimorgans and verrucologen. *C. immitis* has been reported to cause spasms and cramps in sheep and swine. Collectively, these observations further demonstrate the potential for adverse effects from feeding spoiled silages. Additional studies are needed to identify the specific effects of ingestion or inhalation of these and other feed-borne fungi.

**Key words:** dairy, fungi, silage

**T373 Evaluating the inclusion of Met and Lys to mechanically extracted soybean meal with soy gums on the ruminally-undegraded Met and Lys content.** C. A. Macgregor*[1], L. O. Tedeschi[2], and T. K. Miller-Webster[3], *[1]Grain States Soya Inc., West Point, NE, [2]Texas A&M University, College Station, [3]West Virginia University, Morgantown.*

Mechanically-extracted soybean meal (MES) with fresh soy gums (MESG) was compared with MESG with added dl-Methionine and Lysine (MESG-ML) to evaluate the impact of the added Met and Lys on the ruminally-undegraded Met (RUM) and Lys (RUL) using the in situ technique. Dacron bags containing the treatments (TRT), MESG or MESG-ML, were incubated in the rumen of 3 lactating cows for either 4 or 8 h (6 bags/cow/TRT/time) using the simultaneous removal method. Cows were 12, 45, and 222 DIM and milk production was 35.8, 41.3, and 24.4 kg/d, respectively. Met and Lys in the MESG-ML were added to soy gums simultaneously by means of 2 variable augers and mixed into the soy gums in an in-line mixer before soy gums were applied onto the MES at time of manufacture. Met content of MESG and MESG-ML was 0.65 and 0.70% DM and Lys content of MESG and MESG-ML was 2.67 and 2.72% DM, respectively. The RUM and RUL remaining in the Dacron bags at 4 and 8 h were reported as percent of the original sample DM. The statistical analysis was performed as a factorial arrangement (2 TRT × 2 incubation times) in a complete randomized block design, assuming cows as random factors. For Lys, there was no interaction between TRT (MESG vs. MESG-ML) and incubation time ($P = 0.7824$; 2.106 vs. 2.185% at 4 h and 1.707 vs. 1.805% at 8 h, respectively). As expected, the RUL was greater at 4 than at 8 h ($P < 0.0001$; 2.15 vs. 1.76%, respectively). The MESG-ML had a significantly greater RUL than MESG ($P = 0.0145$; 2.0 vs. 1.91%). Similarly, there was no interaction between TRT (MESG vs MESG-ML) and incubation time for Met ($P = 0.7834$; 0.536 vs 0.578% at 4 h and 0.445 vs 0.491% at 8 h, respectively), the RUM at 4 h was greater than at 8 h ($P < 0.0001$; 0.557 vs 0.468%, respectively), and treated MESG with dl-Methionine had greater RUM than control MESG ($P < 0.0001$; 0.534 vs 0.491%, respectively). Our analyses indicated that enriching MESG with dl-Methionine and Lysine using the method described above can increase the content of these 2 amino acids in the ruminally-undegraded protein pool.

**Key words:** amino acid, gums, soybean meal

**T374 Effect of ghrelin on bovine myogenic differentiation.** D. Montoya-Flores*[1,2], O. Mora[1], E. Tamariz[1], L. González-Dávalos[1], A. González-Gallardo[1], A. Antaramian[1], A. Shimada[1], A. Varela-Echavarría[1], and J. L. Romano-Muñoz[2], *[1]Universidad Nacional Autónoma de México, Querétaro, Querétaro, México, [2]Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Colón, Querétaro, México.*

Ghrelin is an acylated hormone, reported to influence food intake, energy metabolism, and reproduction, among others. Ghrelin may also stimulate proliferating myoblast cell differentiation and multinucleated myotube fusion. The aim of this work was to study the effect of human ghrelin (hGHRL) and human ghrelin fragment 1–18 (hGHRL1–18) on myoblast differentiation, measuring the effect of different concentrations on myogenic differentiation by analyzing myogenin expression at the mRNA and protein level. Two types of cells were tested, the cell line i28 obtained from mouse skeletal muscle and primary cultures of bovine myoblasts. Ghrelin and its N-terminal fragment hGHRL1–18 were used at concentrations of 0, 0.01, 0.1, 1, 10, and 100 nM. Treatments were applied to pre-confluent cultures and were maintained during 4 d. Myogenic differentiation of i28 cells was positively affected by hGHRL and hGRHL1–18, starting at a concentration of 0.1 nM ($P < 0.01$). On the other hand, only concentrations of 10 and 100 nM of hGHRL stimulated bovine myoblast differentiation. These results could be attributed to the presence of the mRNA for GHS-R1a and CD36 receptors, in both i28 cells and in bovine myoblasts. Hence, hGHRL might be useful in beef cattle production by promoting muscle differentiation.

**Key words:** ghrelin, bovine, myogenic differentiation

**T375 Essential oil and rumensin affect ruminal fermentation in continuous culture.** D. Ye*[1], S. K. R. Karnati[1], J. L. Firkins[1], M. L. Eastridge[1], and J. M. Aldrich[2], *[1]Ohio State University, Columbus, [2]Provimi-North America, Lewisburg, OH.*

The combination of Rumensin and essential oil could be beneficial for ruminal fermentation by suppressing protozoa and their associated methanogens, while maintaining normal rumen function. The objective of this study was to determine the effects of feeding Rumensin and Cinnagar (essential oil from cinnamon and garlic) in diets on ruminal fermentation characteristics. Four continuous culture fermenters were modified to retain protozoa (slower stirring and a special filter apparatus) and maintained at a liquid dilution rate of 7%/h and solids dilution rate of 5%/h in 4 periods of 10 d each (7 d of adaptation) in a 4 × 4 Latin square design. Four dietary treatments (fed in one meal per day) were arranged in a 2 × 2 factorial: (1) Control diet, 40 g of a 50:50 concentrate: forage (ground alfalfa hay) diet (40% NDF, 17% CP) containing no additive; (2) Rumensin at 11 g/909 kg of DM; (3) Cin-

nagar at 0.0043% (DM basis); and (4) combination of Rumensin and Cinnagar. There were no effects ($P \geq 0.36$) of treatment on concentrations of NH3-N or total VFA. Rumensin (main effect, no interaction) decreased ($P < 0.05$) molar percentages of acetate (62.6 vs. 64.4%) and valerate (1.78 vs. 1.86%); decreased acetate: propionate ratio (2.69 vs. 3.04) but increased ($P < 0.05$) propionate (23.3 vs. 21.3%) and isovalerate (1.94 vs. 1.67%). Rumensin increased ($P < 0.05$) the protozoa generation time (27.6 vs 21.6 h). Cinnagar tended ($P = 0.11$) to increase isovalerate (1.77 vs. 1.67%) and decrease the protozoa counts (14.9 vs. 18.5 × $10^3$/mL). Rumensin and Cinnagar tended ($P = 0.06$) to interact for methane production (29.0, 22.4, 22.0, and 36.9 mmol/d, respectively). Under the conditions of our study, we did not detect an additive response for Rumensin and Cinnagar to decrease protozoal counts or methane production.

**Key words:** Rumensin, essential oil, continuous culture

**T376 Energy value of co-products of bioethanol production: comparison between triticale grain and triticale DDGS.** B. Liu and P. Yu*, *University of Saskatchewan, Saskatoon, Canada.*

The objectives of this study was to compare triticale grain and triticale DDGS on total digestible component nutrient and energy values, estimated using the NRC-2001 summary approach. The triticale grain and triticale DDGS samples were obtained from 3 years. The results showed that triticale DDGS had lower ($P < 0.05$) tdNFC (29.5 vs. 70.1%DM) and higher ($P < 0.05$) tdNDF (12.0 vs. 6.9%DM), tdCP (30.0 vs. 13.3%DM) and tdFA (5.5 vs. 0.5%DM). Triticale DDGS was also lower ($P < 0.05$) in TDN (76.9 vs. 84.5% DM). However there were no significant differences ($P > 0.05$) in DE1X, DE3X (3.34 in triticale DDGS vs. 3.42 Mcal/kg DM in triticale grain), ME3X (2.94 in triticale DDGS vs. 3.01 Mcal/kg DM in triticale grain), NEL3X (1.89 in triticale DDGS vs. 1.92 Mcal/kg DM in triticale grain) for dairy and NEm (2.99 in triticale DDGS vs. 3.06 Mcal/kg DM in triticale grain) and NEg (1.36 in triticale DDGS vs. 1.41 Mcal/kg DM in triticale grain) for beef cattle. The results suggested triticale DDGS as an alternative to triticale grain in dairy and beef diets.

**Key words:** bioethanol co-products, energy values, triticale dried distillers grains with solubles

**T377 Molecular spectral features of functional groups mainly associated with lipid biopolymer in co-products (DDGS) from bioethanol production.** P. Yu* and D. Damiran, *University of Saskatchewan, Saskatoon, Canada.*

To date, there is no study on bioethanol processing-induced changes in molecular structural profiles of lipid biopolymer in DDGS products. The objectives of this study were to (1) determine structural changes that were mainly associated with lipid biopolymer in the co-products that occurred on a molecular level during bioethanol proceesing; (2) quantify the asymmetric and symmetric CH3 and CH2 functional groups, carbonyl ester group and lipid unsaturated groups spectral intensities as well as their ratios, and (3) illustrate the multivariate analyses as a research tool for rapid characterization of biopolymer molecular structures in complex a plant-based feed system. The hypothesis of this study was that bioethanol processing changed the molecular structure profiles in the co-products as opposed to original cereal grains. These changes are highly related to lipid nutrient utilization in animals. The results showed that bioethanol processing had significant effects ($P < 0.05$) on the functional groups spectral profiles which are mainly related to lipid molecular structure in the co-products. The bioethanol processing decreased ($P < 0.05$) the CH3-(a)symmetric to CH2-(a)symmetric ratio, changed ($P < 0.05$) the spectral features of carbonyl C = O ester group and lipid unsaturated group. The results indicated that bioethanol processing changed lipid biopolymer structural conformation and the different types of cereal grains had different sensitivity to the bioethanol processing. The spectral profiles were different between their co-products (wheat DDGS vs. corn DDGS). Different bioethanol plants had different impact on the spectral profiles. The multivariate analyses distinguished the structural differences between the wheat and wheat DDGS and between the corn and corn DDGS. Further study is needed to quantify lipid molecular structural changes in relation to lipid nutrient utilization.

**Key words:** co-products from bioethanol processing, lipid conformation and nutrient availability, molecular structures